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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B), or (C).

Company International Dioxcide, Inc.

Company Agent: Bruce S. Galbraith Date 3/19/87

Title President

Bruce S. Galbraith

GOOD LABORATORY PRACTICE STATEMENT

The submitter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

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STUDIES ON THE PROMOTING AND COMPLETE CARCINOGENIC ACTIVITIES OF SOME OXIDIZING CHEMICALS IN SKIN CARCINOGENESIS

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SUMMARY

Six oxidizing chemicals were tested for promoting and complete carcinogenic activities in skin carcinogenesis using female Sencar mice. In the promotion tests, the chemicals were applied twice a week for 51 weeks after initiation with dimethylbenzanthracene (DMBA). In the tests for complete carcinogenic activities, the chemicals alone were applied for 51 weeks. Benzoyl peroxide was found to be a potent promoter as reported previously. Moreover, possible complete carcinogenic action of this chemical was found in this study. Potential promoting effect was suspected in sodium chlorite. Potassium bromate, ammonium persulphate, hydrogen peroxide and sodium hypochlorite were inactive either as a promoter or a complete carcinogen.

INTRODUCTION

We have recently reported on the carcinogenicity of potassium bromate in male and female F344 rats, which induced renal cell tumors [10,11]. Subsequently, we also found that potassium bromate had a promoting effect on renal tumorigenesis [12]. This chemical has been used widely in the bread-making process for the maturation of flour and in the making of fish-paste products for the improvement of the quality by utilizing its oxidative property [10].

Meanwhile, some of the peroxy compounds which also act as oxidants were reported to have promoting effects in skin carcinogenesis [2]. Benzoyl peroxide, a free radical-generating compound, was proven to be a relatively strong promoter in two-stage skin carcinogenesis [14]. Other peroxides such

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TABLE 1

SKIN TUMOR PROMOTION TESTS IN FEMALE SENCAR MICE INITIATED WITH DMBA

Group	Chemical	No. of effective mice	No. of mice with skin tumors at week				Maximum no. of skin tumors per mouse	No. of mice with squamous cell carcinoma (%)	No. of mice with epidermal hyperplasia (%)
			13	26	38	52			
1	Potassium bromate	19	0	0	0	0	0	0	0
2	Benzoyl peroxide	20	7	16	19	20[a]	15.6[a]	18 (90)[a,b]	20 (100)[a]
3	Ammonium persulphate	20	0	0	2	3	0.3	1 (5)	1 (5)
4	Hydrogen peroxide	20	0	0	2	3	0.6	1 (5)	9 (45)[a]
5	Sodium hypochlorite	20	0	1	3	3	0.2	1 (5)[c]	0
6	Sodium chlorite	20	0	2	6	6	1.0	5 (25)	1 (5)
7	TPA	20	20	20	20	20	40.1[a]	20 (100)[a,c]	20 (100)[a]
8	Acetone	15	0	0	0	0	0	0	0

[a]Significantly different from Group 8 ($P < 0.01$). [b]One lymph node metastasis. [c]Metastasis to lung.

TABLE 2

COMPLETE SKIN CARCINOGENICITY TESTS IN FEMALE SENCAR MICE

Group	Chemical	No. of effective mice	No. of mice with skin tumors at week				Maximum no. of skin tumors per mouse	No. of mice with squamous cell carcinoma (%)	No. of mice with epidermal hyperplasia (%)
			13	26	38	51			
1	Potassium bromate	20	0	0	0	0	0	0	0
2	Benzoyl peroxide	20	0	2	6	8[a]	2.0	5 (25)	6 (30)
3	Ammonium persulphate	20	0	0	0	2	0.2	0	2 (10)
4	Hydrogen peroxide	20	0	0	0	1	0.1	0	1 (5)
5	Sodium hypochlorite	20	0	0	0	0	0	0	0
6	Sodium chlorite	20	0	0	0	0	0	0	0
7	Acetone	15	0	0	0	0	0	0	0

[a] Significantly different from Group 7 ($P < 0.05$).

as lauroyl peroxide and hydrogen peroxide were reported to be weak promoters [9,15].

Based on these findings, we were interested in the possible carcinogenic and promoting activities of some oxidizing chemicals. This paper reports studies on potassium bromate, benzoyl peroxide, ammonium persulphate, hydrogen peroxide, sodium hypochlorite and sodium chlorite which have been used mainly as agents for bleaching of foodstuffs and/or water purification owing to their oxidizing actions. No activity has been demonstrated with benzoyl peroxide either as an initiator or as a complete carcinogen [14]. Therefore we included this chemical in the present experiment to test for its carcinogenic effect at a higher dose and also as a positive control for the promotion test like 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA).

MATERIALS AND METHODS

Female Sencar mice, 4 weeks old, were purchased from Shizuoka Laboratory Center, Shizuoka. Mice were housed, 5 to a plastic hanging cage, with sterilized softwood chips as bedding in a barrier-sustained animal room conditioned at 24 ± 1°C and 55 ± 5% humidity. Food and water were given ad libitum. After 2 weeks of acclimation, dorsal skin of the mice was shaved 48 h before treatment with surgical clippers. Twenty or 15 mice were used in each experimental group. In the experiment for tumor-promoting activity, the mice received a single topical application of 20 nmol of DMBA in 0.2 ml of acetone or acetone only, followed 1 week later by application of chemicals, TPA or acetone for 51 weeks. To test the complete carcinogenic activity, chemicals or acetone only were given topically for 51 weeks. All chemicals were dissolved or diluted in acetone and 0.2 ml was applied to the dorsal skin by automatic pipette twice weekly. The number and diameter of all skin tumors were recorded weekly and body weight was measured monthly. The back of the mice was shaved once a week. At autopsy, the skin and major organs were fixed in 10% buffered formalin and stained with hematoxylin and eosin. The doses for the experiment were determined on the basis of a subacute skin toxicity test for 4 weeks. The sources and doses of chemicals used in this study were as follows: potassium bromate (Matsunaga Chemical Ind., Hiroshima, 40 mg/ml); benzoyl peroxide (Wako Pure Chemical Ind., Ltd., Osaka, 100 mg/ml); ammonium persulphate (Wako Pure Chemical Ind., Ltd., Osaka, 200 mg/ml); hydrogen peroxide (Mitsubishi Gas Chemical, Ltd., 5%); sodium hypochlorite (Kanto Chemical Co., Inc., Tokyo, 1%); sodium chlorite (Wako Pure Chemical Ind., Ltd., Osaka, 20 mg/ml); DMBA (Sigma Chemical Comp., St. Louis, 20 nmol); TPA (Sigma Chemical Comp., St. Louis, 10 μg/ml). Data were analyzed statistically by Fisher's exact probability test and/or the chi-square test.

RESULTS

The results of the promotion tests are presented in Table 1. In the mice treated with benzoyl peroxide, the first skin tumor appeared in week 8. The

incidence of skin tumors was 95% by week 34 and it reached 100% at the end of the experiment. Eighteen of 20 skin tumor-bearing mice developed squamous cell carcinomas. A metastasis to the regional lymph node was found in one case. Although statistically not significant, 30% of the mice treated with sodium chlorite developed skin tumors and 5 out of these 6 mice had squamous cell carcinomas. In this group, the first skin tumor appeared in week 17. In groups applied with ammonium persulphate, hydrogen peroxide or sodium hypochlorite the incidences of skin tumors and squamous cell carcinomas were 15% and 5%, respectively, in week 52. Potassium bromate showed no effect. All the mice treated with TPA after initiation with DMBA as positive controls developed squamous cell carcinomas within 39 weeks. On the other hand, control mice applied with acetone after DMBA-initiation had no skin tumors. Epidermal hyperplasia was evident in mice given benzoyl peroxide, hydrogen peroxide or TPA.

Table 2 shows the results of the tests for complete carcinogenic activity on the skin. The first skin tumor was observed in week 24 in a mouse treated with benzoyl peroxide. At the end of the study, 8 out of 20 mice developed skin tumors by this chemical, of which 5 had squamous cell carcinomas. Epidermal hyperplasia was found in 30% of the mice treated with benzoyl peroxide. Only a few skin tumors were noticed in the mice applied with ammonium persulphate or hydrogen peroxide. No skin tumors developed in the mice applied with potassium bromate, sodium hypochlorite, sodium chlorite or acetone.

In both experiments, the relatively high incidences of adenocarcinomas of the mammary gland and adenomas of the lung and the uterus were observed in all groups. There were no significant differences in the mean survival times and body weights of the mice treated with the chemicals other than TPA in both promotion and complete carcinogenicity tests.

DISCUSSION

There is now accumulating evidence that free oxygen radicals have an important role in our understanding of the mechanism of carcinogenesis, especially in the promotion stage [1,3,16,17]. Benzoyl peroxide, which is used as a generator of free radicals, was noticed because it showed a relatively strong promoting activity in two-stage carcinogenesis in mouse skin [14]. Promoting effect of benzoyl peroxide was evidently reconfirmed in our study and it induced multiple skin tumors and squamous cell carcinomas in 100% and 90% of mice, respectively. Moreover, significant increases in the incidence of skin tumors was observed when benzoyl peroxide alone was applied for 51 weeks. Squamous cell carcinomas developed in 5 out of 8 mice bearing skin tumors. It can be said that benzoyl peroxide has a definite promoter action as well as possible complete carcinogenic property.

It should be stressed that 6/20 (30%) of the mice treated with sodium chlorite had skin tumors in the promotion test and 5 developed squamous

cell carcinomas. To our knowledge, there have been no reports on the promoting activity of sodium chlorite. Toxicologically, sodium chlorite is known to induce oxidative damage to erythrocytes in vitro to deplete glutathione levels with an increase in hydrogen peroxide generation and to produce substantial changes in membrane morphology [6]. This oxidative effect is also reflected in vivo as the induction of hemolytic anemia in rats [5]. Experiments at higher concentrations are to be undertaken in our laboratory to ascertain its potential promoting action.

Potential promoting activity of 10% sodium hypochlorite solution on mouse skin initiated with 4-nitroquinoline 1-oxide was reported [4]. The fact that only 15% of the mice developed skin tumors in the promotion test might be due to the much lower concentration (1%) used in this study.

We applied 5% hydrogen peroxide topically, because solution higher than this caused severe skin toxicity in the preliminary subacute test. This chemical was found to have neither promoting nor complete carcinogenic activity in our study, although it has been reported to have an extremely weak promoting action at higher concentrations [9]. Epidermal hyperplasia, however, was observed in 45% of the mice in the promotion test.

Potassium bromate, found to be a renal carcinogen as well as a renal promoter [10—12], was inactive in this study. This chemical is used also as a neutralizer in permanent wavings that results in contact with the skin [13]. The inactivity of potassium bromate either as a promoter or as a complete carcinogen in skin carcinogenesis seems to show that this chemical is relatively safe for this purpose.

Apart from the study in skin carcinogenesis, hydrogen peroxide was recently found to be carcinogenic, inducing duodenal adenocarcinomas in mice [8], and to have promoting effects in duodenal carcinogenesis in rats [17] upon oral administration. Since peroxy compounds are known to act as skin irritants [9], the carcinogenic and promoting effects of hydrogen peroxide on the duodenum can be considered as the result of local action. In this regard, it will be interesting to study the promoting effect of free radical-generating compounds in organs other than the skin, especially in the gastrointestinal organs.

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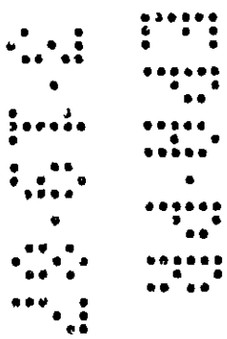

APPENDIX I

The active ingredient in ANTHIUM DIOXCIDE, 5% stabilized chlorine dioxide is sodium chlorite. The more ClO_2 is evolved the lower the pH. The study presented here evaluates sodium chlorite at 20 mg/ml for tumor promoting activity and for complete carcinogenic activity. Our proposed use level of 12.5 - 37.5 mg/liter ppm of total stabilized chlorine dioxide in cutting oils is approximately 1000 times lower concentration.

At the relatively much higher concentration used in this study the conclusions of the painting study are

1) The tumor promoting activity - "Although statistically not significant, 30% of the mice treated with sodium chlorite developed skin tumors and 5 out of 6 mice had squamous cell carcinomas. In this group the first skin tumor appeared in week 17"

2) Complete carcinogenic Activity - "No skin tumors developed in mice applied with potassium bromate, sodium hypochlorite sodium chlorite or acetone".

These above conclusions were made on tests using concentrations of sodium chlorite 1000 times as concentrated as our proposed use.

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